

### Other example of fusion:

**Construct CST-04:** Fusion of the *Neisseria meningitidis* CMP-Neu5Ac synthetase (GenBank #U60146) with Cst-I from *Campylobacter jejuni* (GenBank #BD134499). This construct also includes a Gly-Gly-Gly-Ile linker between the fusion partners and a 6-His tag at the C-terminus.

**Figure 1:** DNA sequence of the fusion gene in CST-04

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ATGGAAAAACAAAATATTGCGGTTATACTTGCGCGCCAAAACCTCCAAAGGATTGCCATTAAAAA
ATCTCCGGAAAATGAATGGCATATCATTACTTGGTCATACAATTAATGCTGCTATATCATCAAA
GTGTTTTGACCGCATAATTGTTTCGACTGATGGCGGGTTAATTGCAGAAGAAGCTAAAAATTTTC
GGTGTCTGAAGTCGTCTACGCCCTGCAGAGCTGGCCTCCGATACAGCCAGCTCTATTTTCAGGTG
TAATACATGCTTTAGAAACAATTGGCAGTAATTCCGGCACAGTAACCCCTATTACAACCAACCAG
TCCATTACGCACAGGGGCTCATATTCGTGAAGCTTTTTCTCTATTTGATGAGAAAATAAAAGGA
TCCGTTGTCTCTGCATGCCCAATGGAGCATCATCCACTAAAAACCCTGCTTCAAATCAATAATG
GCGAATATGCCCCCATGCGCCATCTAAGCGATTTGGAGCAGCCTCGCCAACAATTACCTCAGGC
ATTTAGGCCTAATGGTGCAATTTACATTAATGATACTGCTTCACTAATTGCAAATAATTGTTTTT
TTTATCGCTCCAACCAAACCTTTATATTATGTCTCATCAAGACTCTATCGATATTGATACTGAGC
TTGATTTACAACAGGCAGAAAACATTCTTAATCACAAGGAAAGCGGTGGCGGAATTCTGACAAG
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GGACCTAGCCTAAAAAATATTAATTATAAAAGACTGCCTAGAGAATATGATGTTTTTTAGGTGTA
ACCAGTTTTATTTTGAAGATAAGTATTATTTAGGAAAAAAGATTAAAGCAGTATTTTTTAATCC
TGGTGTCTTTTTTACAACAGTATCACACTGCAAAACAACCTTATACTAAAAAATGAGTATGAAATA
AAAAATATTTTTTTGCTCTACATTTAATTTACCTTTTATTGAAAGCAATGATTTTTTTACATCAAT
TTTATAATTTTTTTCCCGATGCAAAACTTGGCTATGAAGTTATTGAAAACCTTAAAGAATTTTA
TGCTTATATAAAATACAATGAAATTTATTTCAATAAAAGAATTACTTCGGGCGTCTATATGTGT
GCAATTGCTATTGCATTAGGATATAAAACCATCTATTTATGTGGCATTGATTTTTTATGAAGGAG
ATGTTATTTATCCTTTTGAAGCTATGAGTACAAATATAAAAACAATCTTTCCTGGAATAAAAGA
TTTCAAACCTTCAAATTGTCATTCTAAGGAATACGATATAGAAGCATTAAAATTGTTAAATCA
ATATACAAAGTTAATATCTACGCATTGTGTGATGATTCTATTTTGGCAAATCATTTTCCTTTAT
CAATTAATATTAATAACAATTTCACTTTAGAAAATAAGCATAATAATTCTATAAATGATATTTT
ATTGACTGATAATACTCCTGGCGTAAGTTTTTATAAAAATCAACTTAAAGCTGATAATAAAATT
ATGCTTAATTTTTTATAATATTCTTCATTCTAAAGATAATTTAATTAAATTTTTTAAACAAAGAAA
TTGCGGTATTAAAAAAACAAACCACTCAACGAGCTAAAGCAAGAATCCAAAACCATCTATCCTA
TAAACTAGGACAAGCTTTGATTATAAATTCTAAAAGTGTATTAGGTTTTTTTATCTTTACCTTTT
ATAATATTAAGTATCGTTATTTACATAAACAAGAACAAAAGGCTTATAAATTTAAAGTAAAGA
AAAATCCAAATTTAGCTTTACCTCCTTTAGAACTTATCCTGATTATAATGAAGCTTTAAAGAA
AAAAGAATGTTTTACTTATAAATTAGGAGAAGAATTTATAAAAGCTGGTAAGAATTGGTATGGG
GAGGGGTATATCAAATTTATATTCAAAGATGTTCCCTAGGTTGAAGAGAGAGTTTGAGAAAGGGG
AACATCACCACCATCACCCTAATGA
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**Figure 2:** Amino acid sequence of the fusion protein in CST-04.

## EXHIBIT C

MEKQNI AVILARQNSKGLPLKNLRKMNGISLLGHTINAAISSKCFDRIIVSTDGGLIAEEAKNF  
 GVEVVL RPaelASDTASSISGVIHALETIGSNSGTVTLLQPTSPLRTGAHIREAFSLFDEKIKG  
 SVVSACPM EHHPLKTL LQINNGEYAPMRHLS DLEQPRQQLPQAFRPN GAIYINDTASLIANNCF  
 FIAPTKLYIMSHQDSIDIDTELDLQQAENILNHKESGGGILTRTRMENELIVSKNMQNII IAGN  
 GPSLKNIN YKRLPREYDVFR CNQFYFEDKY YLGKKIKAVFFNPGVFLQQYHTAKQLILKNEYEI  
 KNIFCSTFNLPFIESNDFLHQFYNFPPDAKLG YEVIENLKEFYAYIKYNEIYFNKRITSGVYMC  
 AIAIALGYKTIYLCGIDFYEGDVIYPFEAMSTNIKTIFPGIKDFKPSNCHSKEYDIEALKLLKS  
 IYKVNIYALCDDSI LANHFPLSININNNFTLENKHNN SINDILLTDNTPGVSFYKNQLKADNKI  
 MLNFYNILH SKDNLIKFLNKEI AVLKKQTTQRAKARIQNHL SYKLGQALIINSKSVLGFLSLPF  
 IILSIVISHKQEQKAYKFKVKKNPNLALPPLETYPDYNEALKEKECFTYKLGEEFIKAGKNWYG  
 EGYIKFIFKDVPR LKREFEKG EHHHHH

**Experimental evidence for activity of both fusion partners:**

**Table 1:** Activity measured in the various fractions of an extract<sup>1</sup> of *E. coli* AD202/CST-04 using a direct assay for  $\alpha$ -2,3-sialyltransferase<sup>2</sup> activity and a coupled assay<sup>3</sup>.

Fraction	Total activity Direct assay <sup>2</sup> (mU) <sup>4</sup>	Total activity Coupled assay <sup>3</sup> (mU)
15,000 rpm supernatant	2,289.6	1,612.0
15,000 rpm pellet	3,877.2	7,509.6
55,000 rpm supernatant	134.8	177.2
55,000 rpm pellet	895.2	897.2

<sup>1</sup> CST-04 was transformed in *Escherichia coli* AD202. A 200 mL culture was grown at 37°C and induced with 0.5 mM IPTG. The culture was grown for a total of 24 h and the cell extract was made using an Emulsiflex homogenizer. The extract was centrifuged at 15,000 rpm and the resulting supernatant was centrifuged at 55,000 rpm.

<sup>2</sup> The direct assay for  $\alpha$ -2,3-sialyltransferase activity was performed using 1 mM Lac-FEX, 0.2 mM CMP-Neu5Ac, 10 mM MgCl<sub>2</sub> and 50 mM Hepes pH 7.5.

<sup>3</sup> The coupled assay for CMP-Neu5Ac synthetase and  $\alpha$ -2,3-sialyltransferase activities was performed using 1 mM Lac-FEX, 3 mM CTP, 3 mM Neu5Ac, 0.2 mM DTT, 10 mM MgCl<sub>2</sub> and 50 mM Hepes pH 7.5. The reactions were performed at 37°C for 5 min.

<sup>4</sup> A milli-unit (mU) of activity is defined as the amount of enzyme that converts one nanomol of product per minute.

### Other example of fusion: CST-02

**Construct CST-02:** Fusion of the *Neisseria meningitidis* CMP-Neu5Ac synthetase (GenBank #U60146) with truncated Cst-I (328 aa) from *Campylobacter jejuni* (GenBank #BD134499). This construct also includes a Gly-Gly-Gly-Ile linker between the fusion partners and a 6-His tag at the C-terminus.

**Figure 1:** DNA sequence of the fusion gene in CST-02

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ATGGAAAAACAAAATATTGCGGTTATACTTGCGCGCCAAAACCTCCAAAGGATTGCCATTAAAAA
ATCTCCGGAAAATGAATGGCATATCATTACTTGGTCATACAATTAATGCTGCTATATCATCAAA
GTGTTTTGACCGCATAATTGTTTCGACTGATGGCGGGTTAATTGCAGAAGAAGCTAAAAATTTTC
GGTGTCTGAAGTCGTCTACGCCCTGCAGAGCTGGCCTCCGATACAGCCAGCTCTATTTTCAGGTG
TAATACATGCTTTAGAAACAATTGGCAGTAATTCCGGCACAGTAACCCTATTACAACCAACCAG
TCCATTACGCACAGGGGCTCATATTCGTGAAGCTTTTTTCTCTATTTGATGAGAAAATAAAAGGA
TCCGTTGTCTCTGCATGCCCAATGGAGCATCATCCACTAAAAACCCTGCTTCAAATCAATAATG
GCGAATATGCCCCCATGCGCCATCTAAGCGATTTGGAGCAGCCTCGCCAACAATTACCTCAGGC
ATTTAGGCCTAATGGTGCAATTTACATTAATGATACTGCTTCACTAATTGCAAATAATTGTTTT
TTTATCGCTCCAACCAAACCTTTATATTATGTCTCATCAAGACTCTATCGATATTGATACTGAGC
TTGATTTACAACAGGCAGAAAACATTCTTAATCACAAGGAAAGCGGTGGCGGAATTCTGACAAG
GACTAGAATGGAAAATGAACTCATTGTTAGTAAAAATATGCAAAATATAATCATAGCAGGAAAT
GGACCTAGCCTAAAAAATATTAATTATAAAAGACTGCCTAGAGAATATGATGTTTTTAGGTGTA
ACCAGTTTTATTTTGAAGATAAGTATTATTTAGGAAAAAAGATTAAAGCAGTATTTTTTAATCC
TGGTGTCTTTTTTACAACAGTATCACACTGCAAAACAACCTTATACTAAAAAATGAGTATGAAATA
AAAAATATTTTTTGTCTCTACATTTAATTTACCTTTTATTGAAAGCAATGATTTTTTACATCAAT
TTTATAATTTTTTCCCCGATGCAAAACCTTGGCTATGAAGTTATTGAAAACCTTAAAGAATTTTA
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GCAATTGCTATTGCATTAGGATATAAAACCATCTATTTATGTGGCATTGATTTTTTATGAAGGAG
ATGTTATTTATCCTTTTGAAGCTATGAGTACAAATATAAAAAACAATCTTTCCTGGAATAAAAGA
TTTCAAACCTTCAAATTGTCATTCTAAGGAATACGATATAGAAGCATTAAAATTGTTAAATCA
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CAATTAATATTAATAACAATTTCACTTTAGAAAATAAGCATAATAATTCTATAAATGATATTTT
ATTGACTGATAATACTCCTGGCGTAAGTTTTTTATAAAAATCAACTTAAAGCTGATAATAAAATT
ATGCTTAATTTTTTATAATATTCTTCATTCTAAAGATAATTTAATTAAATTTTTTAAACAAAGAAA
TTGCGGTATTAAAAAAACAAACCACTCAACGAGCTAAAGCAAGAATCCAAAACCATCTATCCTA
TAAACTAGGACAAGCTCATCACCACCATCACCCTAATGA
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**Figure 2:** Amino acid sequence of the fusion protein in CST-02.

MEKQNI AVILARQNSKGLPLKNLRKMNGISLLGHTINAAISSKCFDRIIVSTDGGLIAEEAKNF  
GVEVVL RPaelASDTASSISGVIHALETIGSNsgTVtLLQPTsPLRTGAHIREAFSLFDEKIKG  
SVVSAC PMEHhPLKtLLQINNGEYAPMRHLSdLEQPRQQLPQAFRPNGAIYINDTASLIANNCF  
FIAPTKLYIMSHQDSIDIDTELDLQQAENILNHKESGGGILTRTRMENELIVSKNMQNI I IAGN  
GPSLKNIN YKRLPREYDVFRcNQfYFEDKYyLGKKIKAVFFNPGVFLQqYHTAKQLILKNEYEI  
KNIFCSTFNLPFIESNDfLHQfYNFFPDAKLGYEVIENLKEFYAYIKYNEIYFNKRITSGVYMC  
AIAIALGYKTIYLCGIDfYEGDVIYPFEAMSTNIKTIFPGIKDFKPSNCHSKEYDIEALKLLKS  
IYKVNIYALCDDSiLANHFPLSiNINNNfTLENKHnNSINDiLLTDNTPGVsFYKNQLKADNKI  
MLNFYNI LHSKDNLIKfLNKEIaVLKKQTTQRAKARIQNHLSYKLGAHHHHHH